2020

Evidence of Local Adaptation to Climate in an Invasive Ectotherm: A Study on the Eurasian Gypsy Moth (Lymantria dispar) in North America

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Evidence of Local Adaptation to Climate in an Invasive Ectotherm: A Study on the Eurasian Gypsy Moth (*Lymantria dispar*) in North America

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Environmental Studies at Virginia Commonwealth University

by

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July 2020

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ABSTRACT

Invasive species exist in nearly every ecosystem. Improving our understanding of the mechanisms that shape the ability of these organisms to physiologically cope with their surroundings will be crucial to predicting the impacts of future biological invasions. The gypsy moth (*Lymantria dispar*) has been expanding its range across North America over the last 100+ years since its arrival in Medford, Massachusetts. This study quantifies upper thermal tolerance across 8 populations of *L. dispar* in North America and seeks to determine if signals of local adaptation to climate across a latitudinal gradient may be present. Upper thermal tolerance for each population was determined through the use of a temperature controlled water bath and involved both acute and extended heat exposure. Results from linear regression analysis and generalized linear modeling of temperature ramping trials indicated that the upper thermal limit (UTL) for each population was highly correlated with 30 year annual and seasonal temperature averages, as well as latitude. Linear and logistic regression analyses of extended soak trials showed no significant relationships between percent population survival at 43°C and 30 year seasonal/annual temperature averages, latitude, weight or elevation. Although the results were not significant, the trends from this analysis were generally in the same direction as those observed in the ramp trials. Based on these results, we conclude that variation among populations

in upper thermal tolerance is closely related to climate, which is consistent with the hypothesis of local adaptation to temperature as part of the invasion process.

INTRODUCTION

Understanding the processes through which the spread of invasive species takes place is of paramount importance to ecology, economics, and society in general (Gaston 2003; Sexton et al. 2009; Faske et al. 2019). Non-native species have the potential to alter the dynamics that exist in native ecosystems both aquatic and terrestrial (Chown et al. 2007; Tobin et al. 2016) in addition to causing potential economic harm. As these non-native organisms expand within an ecosystem they encounter new landscapes, divergent climates, resource limitations, and interspecies interactions that will ultimately determine their distribution and abundance. The impact of these factors on the fitness of individuals will determine population dynamics at range edges, and therefore the rates and directions of invasive spread (Gaston 2003).

Populations at range edges are often characterized by low population density and may experience novel and possibly extreme environmental conditions compared to those in the established range (Sexton et al. 2009; Tobin et al. 2014; Grayson & Johnson 2018). How organisms cope with these conditions contributes to the growth and spread of invasive species as populations either acclimate, evolve, and expand or are unsuccessful and remain either static or shrinking (Grayson & Johnson 2018).

The processes that take place to shape populations at range edges combined with the potential impacts of global climate change are still not fully understood. Despite an abundance of knowledge in the general field of thermal ecology (see review by Angilletta, 2009), a knowledge gap still exists surrounding the effects of thermal physiology on biological invasions and range

dynamics. This is a crucial area of study as we currently live in a world where invasive species exist on every continent due to increased transferability of organisms from global trade and travel (Bigsby et al. 2010). These factors have aided in pushing invasion dynamics to the forefront of ecological research. Developing an understanding of the ability of invasive species to adapt to temperature will improve our ability to predict invasive spread and how organisms will react to changes in climate. Overall, improving our knowledge of the processes that shape range dynamics will be imperative for making informed management and conservation decisions on a planet that is rife with environmental change.

The spread of *L. dispar* across North America is potentially the most well documented biological invasion in the world, with records stretching back nearly 100 years due to the USDA's involvement in attempting to contain the invasion since 1905 (Weseloh 2003). This species is capable of large-scale defoliation events when populations increase to an outbreak level resulting in significant negative ecological and economic impacts (Bjornstad et al. 2010). Early efforts at containment of *L. dispar* were primarily biological control methods such as importing, rearing, and releasing insect parasitoids from Europe and Japan (Weseloh 2003). Over a 25-year period, 47 different species would be released in an attempt to mitigate the invasive spread, followed by the implementation of physical barriers, prescribed burnings, and airborne pesticides; however, these efforts proved to be largely ineffective (Weseloh 2003).

Lymantria dispar is a generalist feeder of over 300 different species of hardwood trees and shrubs and has expanded its range by approximately 900,000 km^2 since its North American debut in Medford, Massachusetts circa 1869 (Grayson & Johnson 2018). This expansion has taken place across nearly 20° of latitude, which encompasses a wide range of climates, forested landscapes, and elevations across North America. As a result, an estimated 78 million acres of

forest have been defoliated by *L. dispar* since 1970 with many millions of dollars having been spent on attempts to contain the moth (Tobin et al. 2012). *Lymantria dispar* was originally brought to the US by the French artist and amateur entomologist Etienne Leopold Trouvelot (Weseloh 2003). Trouvelot settled in Medford, from which gypsy moth spread across the entire Northeastern US and into parts of the Midwest and as far south as coastal North Carolina (Grayson & Johnson 2018). This however is only a third of the theoretically suitable range of *L. dispar* in the US (Grayson & Johnson 2018). Early models of the expected spread of *L. dispar* in the US predicted its range extending as far south as northern Florida; however, the actual observed spread of this species has its southernmost extent in coastal North Carolina, with higher rates of expansion in high elevation and high latitude locations (Fig 1). This suggests that a physiological limitation such as a thermal threshold may have been reached in the south, which is limiting the spread. Future changes in climate combined with local adaptation to temperature could further alter the range and spread of this invasive ectotherm as temperatures continue to rise over the next several decades.

Cases of relatively rapid adaptive physiological change related to climate have been observed in a variety of other invasive insect species (Chown et al. 2007; Preisser et al. 2008; Huey & Pasqual 2009). We hypothesize that local adaptation in response to temperature is occurring in *L. dispar* across the climatic gradient of its invasive range. Specifically, we predict that populations from higher elevations and latitudes (*i.e.*, colder climates) will have lower tolerance to high temperatures than populations from lower elevations and latitudes *(i.e.*, warmer climates). The goal of this study is to quantify upper thermal tolerance across populations of *L. dispar* to test for signals of local adaptation to climate. This research is a component of a larger National Science Foundation funded project that aims to determine how physiological

performance and local adaptation contribute to invasion front dynamics and how they interact with climate to determine the spatial spread of *L. dispar*. This is a multifaceted project that seeks to compare the physiology of populations across the invasion front, predict performance under current and future climates, and determine how thermal performance interacts with climate to influence invasive spread.

MATERIALS AND METHODS

Egg masses for each population were collected in the field by the Parry Lab at SUNY-Syracuse in 2016/2017 and sent to VCU for rearing and experimentation as part of the larger National Science Foundation project. These populations represent regions across the invasion front and within the established range along a latitudinal gradient including: North Carolina, Southwest Virginia, West Virginia, New York, Massachusetts, Wisconsin, and Southern Canada (Fig. 1). Standard approaches to assess the thermal tolerance of ectotherms were used. The first approach (temperature ramping) involved heating the organism until a pre-determined response was observed (Cowles et al. 1944; Lutterschmidt et al.1997; Terblanche et al, 2007). The temperature at which this response occurred was used as a measure of the upper thermal limit (UTL). There are several different commonly used responses such as the onset of spasms (loss of controlled muscular functions) and knockdown (induced heat coma characterized by lack of righting response). Due to the large number of larvae observed during the experiment, knockdown was chosen as the pre-determined response for this study as it is more easily observable than the onset of spasms. The second approach (temperature soak) involved measuring the response to extended exposure to an extreme temperature based on how well

populations survived (Sinclair et al. 2012). By using these two different but complementary approaches to assessing thermal tolerance, I hoped to achieve greater confidence in my findings.

To provide specimens for the experiments, N=50 *L. dispar* larvae from each of the eight populations were group reared inside of a temperature-controlled incubator (Percival I-22VL) at 25°C until they reached the third instar. Analysis occurred at the third instar because this is the approximate midpoint of the larval period and because they are large enough to be easily observed in the experiments. Larvae from each population were weighed, individually placed into glass vials, and allocated to one of the two different experimental treatments (n= 20 per population for the ramp trials, n=10 per population for the soak trials at each of three temperature extremes).

For the ramping trials, a potential issue with this approach is the possibility of a lag time between the temperature of the water bath, air temperature inside the vial, and body temperature of the organism (Terblanche et al. 2007; Agosta et al. 2018). For the third instar larvae used in this experiment, previous studies indicate little to no issue with lag at a ramp rate of 0.25 °C per minute (Terblanche et al. 2007; Agosta et al. 2018). Thus, lag effects were assumed to be negligible or nonexistent in this study and were not considered further.

Measuring Upper Thermal Limits

For the ramping procedure, N=20 larvae for each population were placed individually in numbered glass vials and submerged in a programmable temperature-controlled water bath (Huber CC-118A with Pilot One) at 25°C for fifteen minutes to allow the air and body temperature of the larva inside the vial to equilibrate (Agosta et al. 2018). 25°C was chosen as the starting point due to its equitability for *L. dispar* larvae and to limit the time it took to

conduct ramping trials. Following the equilibration period, temperature was increased incrementally by 0.25°C per minute until larvae entered into a heat coma (termed a "knockdown" point) following the onset of spasms. This point is identified through observations such as the loss of a "righting response" (returning to an upright position after being flipped over) and the loss of the ability to grip a surface (Agosta et al. 2018). The temperature at which this knockdown point was reached was then recorded as the UTL.

Measuring Survivorship at Temperature Extremes

For the soak procedure, larvae were placed individually inside of numbered glass vials and submerged in a temperature-controlled water bath. Groups of N=30 larvae from each population were subjected to three different temperatures (43°C, 44°C, and 45°C) for a time of one hour (N=10 per temperature, per population). Following the soak treatment, larvae were placed into individual rearing cups for up to 24 hours to observe if there was successful recovery from the induced heat coma. Individuals were assessed on whether or not they survived the exposure. Starting at 43°C for the soak trials was chosen based on preliminary work conducted using a laboratory strain of *L. dispar*. Using trial and error across a variety of temperatures, 43°C was determined to be a good starting point based on high variability in survivorship among populations. Although trials were also conducted at 44°C and 45°C , only results from 43°C were analyzed due to extremely high mortality in all populations at the higher temperatures.

Characterizing Local Climates

Climate variables for each population were acquired from the National Centers for Environmental Information and included climate normals for temperature measured as 30-year

averages (1981-2010). Weather stations were accessed via the search tool on NOAA's website and were selected based on proximity to the study site where *L. dispar* eggs were collected. The 30-year climate normals used in this study included the average, maximum, and minimum annual, spring, and summer temperatures for each study site, in addition to their latitude and elevation. Fall and winter climate normals were not included in the study because third instar *L. dispar* larvae do not typically occur during those seasons. Latitude and elevation are commonly assessed in studies on thermal tolerance due to their relationship with climate (Addo-Bediako et al. 2000; DeLong et al. 2018; Howard-Williams et al. 2006) and will therefore be included in this study as geographic proxies for local climates.

Statistical Analysis

The data used in this study were found to be normally distributed based on Q-Q plots. For the ramp trials, the data were first analyzed using linear regression to test for relationships between UTL and individual climate variables. UTL values were averaged for each population to determine a population-level UTL which was then used for statistical analysis. Linear regression was used to assess population-level UTL against 30-year temperature averages for each study site. Following these analyses, a variety of generalized linear models were created in order to determine which temperature variables were best at explaining variation in UTL's among populations. This was done through an iterative process of adding and removing variables based on their p-values. Models were assessed based on AIC score with the best-predicting model having the lowest observed AIC score.

For the soak trials, survivorship at 43°C for each population was converted to a percentage for statistical analysis. Both linear and logistic regression were used to analyze the

soak trial data. Typically, linear regression is not used with percentages; however, when the data are within the range of 0.2-0.8, linear regression is justified (Long 1997). GLM's were not created for the soak data due to a lack of statistically significant relationships from the linear regression analysis. All statistical analyses were conducted using the open source statistical software RStudio (Version 1.1.456). All tests were considered significant at $p < 0.05$. All means are reported with ±1 standard error.

RESULTS

The average UTL of *L. dispar* larvae ranged from 46.7°C - 47.14°C and the overall mean UTL across all populations included in this study was 46.93°C $\pm$ 0.04, which is well within the range of values found for other insects (Hoffman et al. 2013; Agosta et al. 2018). The average survivorship of *L. dispar* larvae at 43°C ranged from 30% - 80% among populations with an overall mean across populations of 52 $\pm$ 0.07%.

Weight

Weight was not a significant predictor ($p > 0.05$) of average population UTL or average population survivorship at 43°C (Fig. 2), therefore this variable was not considered in subsequent analyses.

Latitude and Elevation

Latitude was a significant predictor of population UTL ($R^2 = 0.56$, p-value = 0.02) with populations from higher latitudes generally having lower thermal tolerance than those from lower latitudes (Fig. 3a). A similar relationship with latitude was found for survivorship at 43°C

(Fig. 3c), however, it was not statistically significant (R^2=0.37, p-value =0 .088). Elevation was not a significant predictor for either response variable (Fig. 3b,d) and therefore was not considered in further analyses.

30-Year Temperature Averages – Ramp Treatment

Results from linear regression analyses of 30-year temperature averages from each study site showed statistically significant relationships between all variables and population UTLs (Fig. 4), with the exception of 30-year average maximum summer temperature (p-value > .05; Fig. 4h). The final GLM based on ramp trial results contained the variables for 30-year average minimum spring temperatures and 30-year average minimum summer temperatures (Table 1). This model had low residual deviance (0.0261) suggesting that the model fits the data reasonably well (Pierce & Schafer 1986) and an AIC score of -15.088 (range of all models: -9.9216 - -15.088). The second best model included the variables for 30-year average minimum spring temperatures as well as latitude (AIC = -14.677), and the third best model included 30-year average minimum spring temperatures, 30-year average minimum summer temperatures, and latitude (AIC = -14.174).

30-Year Temperature Averages – Soak Treatment

Results from linear regression analyses of 30-year temperature averages from each study site indicated no statistically significant relationships (all p's > 0.05) between any of the variables and survivorship at 43°C (Fig. 5). However, while the findings were not significant, the relationships exhibited trends in the same direction as those from the ramp treatment analysis.

Logistic regression was also utilized to analyze the survivorship data and also showed that the results were not significant ($p > 0.05$).

DISCUSSION

The strong linear relationships shown between the variables in the ramp trials along with similarly observed trends from the soak trials (albeit not statistically significant) indicate that variation among populations in upper thermal tolerance of *L. dispar* larvae is related to climate, specifically temperature. This conclusion is corroborated by the models produced by GLM, which indicate the variables that best explain the variation in UTL are 30-year averages of minimum spring and summer temperatures, in addition to latitude. The relationships observed in both the ramp and soak trials indicate that populations occurring at higher latitudes generally have lower UTL's and survivorship at an extremely high temperature (43°C) compared to populations from lower latitudes. The presence of this relationship with latitude for both response variables, while not significant for the survivorship data, suggests that the thermal tolerance of *L.dispar* is evolving across the invasion front as it encounters divergent climates. Overall these data support the prediction that populations at higher latitudes have lower thermal tolerance than those occurring at lower latitudes, which is consistent with the hypothesis of thermal adaptation.

While the relationships between UTL and temperature variables were fairly strong, values for UTL across populations differed across a small range of 0.4°C. This small range of observed values across populations is consistent with results from other intra-specific studies, as well as the general pattern showing less variation among ectotherms in upper as opposed to lower thermal limits, possibly because the evolution of upper thermal tolerance is more

constrained (Terblanche et al. 2006; Addo-Bediako et al. 2000; Angilletta 2009; Jensen et al. 2019). In fact, a recent study shows evidence that heat tolerance has evolved more slowly than cold tolerance in vertebrates (Qu Yan-Fu et al. 2020). Another recent study on the arthropod *Orchesella cincta* found that thermal tolerance differed among populations across a small range of approximately 0.5°C (similar to this study) and cited latitude as the primary predictor of this variation (Jensen et al. 2019). Heat tolerance for this species was higher in populations occurring in warm, low-latitude areas whereas the most cold-tolerant populations were found in cooler, high-latitude regions (Jensen et al. 2019).

A study conducted on the ectotherm *Glossina pallidipes* also showed a small range of UTL values (approximately 1°C) amongst populations (Terblanche et al. 2006). Although variation in UTL's found in this study was very small, the values were strongly related to climate variables and latitude which shows a signal that is consistent with local adaptation to temperature. Additionally, the univariate relationships assessed in the ramp trials via linear regression showed high R^2 values (> 0.5) for nearly all of the variables related to climate, similar to the relationships found here for *L. dispar*. The strength of these relationships suggests that, despite the small range of variation observed, thermal adaptation may still be occurring in response to local climates. This could take place either as a result of selection on the whole thermal performance curve and/or as direct selection on upper thermal tolerance specifically. For invading species like *L. dispar*, it is possible that populations are in the early stages of thermal adaptation, and that the range of variation in thermal tolerance may become larger over time.

While the relationships observed in the ramp portion of this experiment exhibited strong patterns in the expected direction for UTL and are consistent with other studies conducted on various ectotherm species, the soak trial portion revealed no statistically significant relationships.

However, the relationships observed for survivorship at 43°C did show trends that were similar to the UTL data, suggesting that more study on this dimension of thermal tolerance may be warranted.

The lack of a relationship between UTL and weight somewhat contradicts previous studies on ectotherms that have shown correlations between body size and heat tolerance (Klockman et al. 2017; Oberg et al. 2012; Brans et al. 2017). However, there have also been studies that have shown no correlation (Nyamukondiwa & Terblanche. 2009), which is consistent with the results seen here. Contrasting findings such as these suggest that body size may affect thermal tolerance for some species or life stages but not others, or that the range of weight variation was too small in this study (all 3rd instar larvae) to have a significant effect. Additionally, elevation was not found to be a significant predictor of thermal tolerance in *L. dispar*. The lack of statistical significance for this variable may potentially be explained by spatial scale and gene flow. Significant effects of latitude but not elevation suggests that latitude is at a large enough spatial scale so that the effects of gene flow on evolution are minimal, whereas elevation occurs at a much smaller scale where gene flow is large enough to impede local adaptation.

Populations of *L. dispar* occurring at the study sites from southern latitudes within this system have already been shown to have more resilience to temperature extremes than populations occurring at northern latitudes, however this increased heat tolerance may come at the cost of overall performance (Thompson et al. 2017). Southern populations were found to have higher thermal tolerance yet exhibited lower pupal mass and development time (when reared at warm temperatures) compared to northern populations; suggesting that high thermal tolerance comes at the cost of general physiological performance (Thompson et al. 2017).

Additionally, *L. dispar* may be more susceptible to the effects of extreme temperatures based on the life stage it is in (Banahene et al. 2018). Exposure to high temperatures, especially for extended periods of time, has detrimental effects on third-instar survival, pupal growth, and development (Banahene et al. 2018). Another recent study based on genomic analysis provides evidence that evolutionary divergence has occurred among populations of *L. dispar* in North America (Friedline et al. 2019). This provides further evidence that thermal adaptation may be occurring across populations of *L. dispar* along the latitudinal gradient of its invasive range. As temperatures continue to warm from climate change, it is expected that the range, distribution, and spread of this species will be altered as a result. Better understanding the relationships between thermal tolerance and climate will help to prepare for these ecological changes and to make informed management decisions for the future.

Signals of local adaptation to temperature have also been observed in some marine ectotherms. For example, researchers studying the tide pool copepod *Tigriopus californicus* have found signals of local adaptation to both temperature and salinity across a latitudinal gradient. Species from 14 populations across the North American coast were collected from the field and reared in the laboratory for 2 generations in temperature controlled incubators. The organisms were then subjected to temperature ramping trials in which they were placed in a temperature controlled sea water environment at 20°C and temperature was increased at a specified rate until mortality occurred. Results showed strong relationships between latitudinal position of the population and tolerance to both salinity and heat. Southern populations of *T. californicus* were consistently more tolerant to high temperatures and high salinity whereas northern populations exhibited low salinity tolerance (Leong et al. 2017). Strong evidence for local adaptations to climate was detected within a distance as small as 5.6km between populations. These patterns are

likely driven by high variability in geographic formation, “coastline orientation, wave energy, rock type, and pool depth” between tide pools (Leong et al. 2017).

Although it may be ecologically uncommon for organisms to experience extreme temperatures outside of their thermal limits, research has shown that even occasional exposure to extreme temperatures can impact the distribution of ectotherms (Hance et al. 2007). Furthermore, while these extreme temperatures may be relatively uncommon in nature today, climate change may result in more frequent exposure to the extremes. The frequency of exposure to extreme temperatures experienced by populations in this study may be best understood through a comparison of UTLs with historical maximum daily air temperatures from each of the study sites where populations originated (Agosta et al. 2018). This comparison is in the process of being made and will be added as a supplement to this study in the future to provide better ecological context for the results.

This project was somewhat limited in the number of populations that could be included by the limited hatching success of some egg masses. While the populations used in this study all had successful hatchings, several other populations that would otherwise have been included either had poor hatching numbers or simply did not hatch at all. The experiments themselves were also quite time consuming with each ramping trial lasting approximately 80 minutes and each soak trial lasting an hour per population. This lengthy span of time coupled with the limitation of conducting trials only at the third instar life stage left a relatively small window to conduct multiple trials of each treatment for each population. While populations were hatched at staggered intervals in an attempt to address this issue, this interval should be widened for future studies to give the researcher more time to conduct multiple trials. Following up on this project, I would also change the range of temperatures that were used for the soak trials due to the

extremely high mortality observed at 44°C and 45°C. It would likely be more beneficial to conduct these trials over a wider range with 43°C being the highest value to better see the effects of increased extended exposure to extreme temperature.

In conclusion, the results show that the UTL of *L. dispar* populations is closely related to climate, which is consistent with the hypothesis of thermal adaptation occurring across a latitudinal gradient. While the range of values for UTL was small (0.4°C), it was similar to the range of values found in other studies (Terblanche et al. 2006; Addo-Bediako et al. 2000; Angilletta 2009; Jensen et al. 2019). Additionally, all of the relationships to climate variables observed in the ramp trials (with the exception of 30-year average max summer temperature) were found to be significant. Although results from the soak analysis were not significant, the observed trends were similar to those observed in the ramp analysis, suggesting that further investigation in to the effects of extended exposure to extreme temperatures may be warranted (and see Thompson et al. 2017).

Quantifying thermal tolerance in *L. dispar* is only one step in understanding all of the processes at play in determining how this invasive species will spread in the future; however, it is more relevant today possibly than ever as we face a changing climate. Better understanding these processes will be crucial in forming management strategies that will help to prevent further ecological and economic harm. Further studies in this system will help to create a better understanding of biologic invasions as a whole through illuminating some of the physiological processes that organisms undergo when they face environmental change.

ACKNOWLEDGEMENTS

I would like to thank my advisor Dr. Salvatore Agosta for accepting me into the lab and giving me the opportunity to conduct this research. He has always provided me with guidance when necessary and has been a truly invaluable mentor throughout this process. I would also like to thank my lab mate Sean Powers for always being willing to assist me when it was necessary, even in the midst of doing his own PhD research. I thank Dr. Karen Kester for agreeing to be on my committee and allowing me to intern in her lab prior to my acceptance to this graduate program. I also thank Jessica Johnston who was the lab manager in Dr. Kester's lab when I began my internship; her guidance and friendship helped me immensely in the beginning days of graduate school. Many thanks to Kristine Grayson and Lily Thompson from the Grayson lab at University of Richmond for their help in producing diet for the gypsy moth larvae, teaching me how to make the diet as well, and for welcoming me onto the NSF project. I thank Dr. James Vonesh for his instruction and guidance throughout my time in the program which have pushed me to become a better researcher. I also thank Dr. Derek Johnson for agreeing to be a part of my committee and his valuable feedback on my research. Thanks to the Parry lab for providing us with the gypsy moth egg masses, without which none of this research would be possible. I am truly grateful to all of the aforementioned individuals and cannot thank them enough for the roles they all have played throughout my graduate career; I have enjoyed working with you all. This work was supported by the National Science Foundation under Grant DEB 1702701 under the Macrosystems Biology Program.

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Table 1: GLM results examining relationships between UTL and climate variables.

Variables	AIC Score	Residual Deviance	Equation
30 year average minimum spring temperatures, 30 year average minimum summer temperatures	-15.088	0.0261	y = 47.12 + .0464x -.0229x
30 year average minimum spring temperatures, Latitude	-14.677	0.0275	y = 46.90 + .0273x - .0017x
30 year average minimum spring temperatures, 30 year average minimum summer temperatures, Latitude	-14.174	0.0228	y = 46.97 + .1216x - .0942x + .0253x

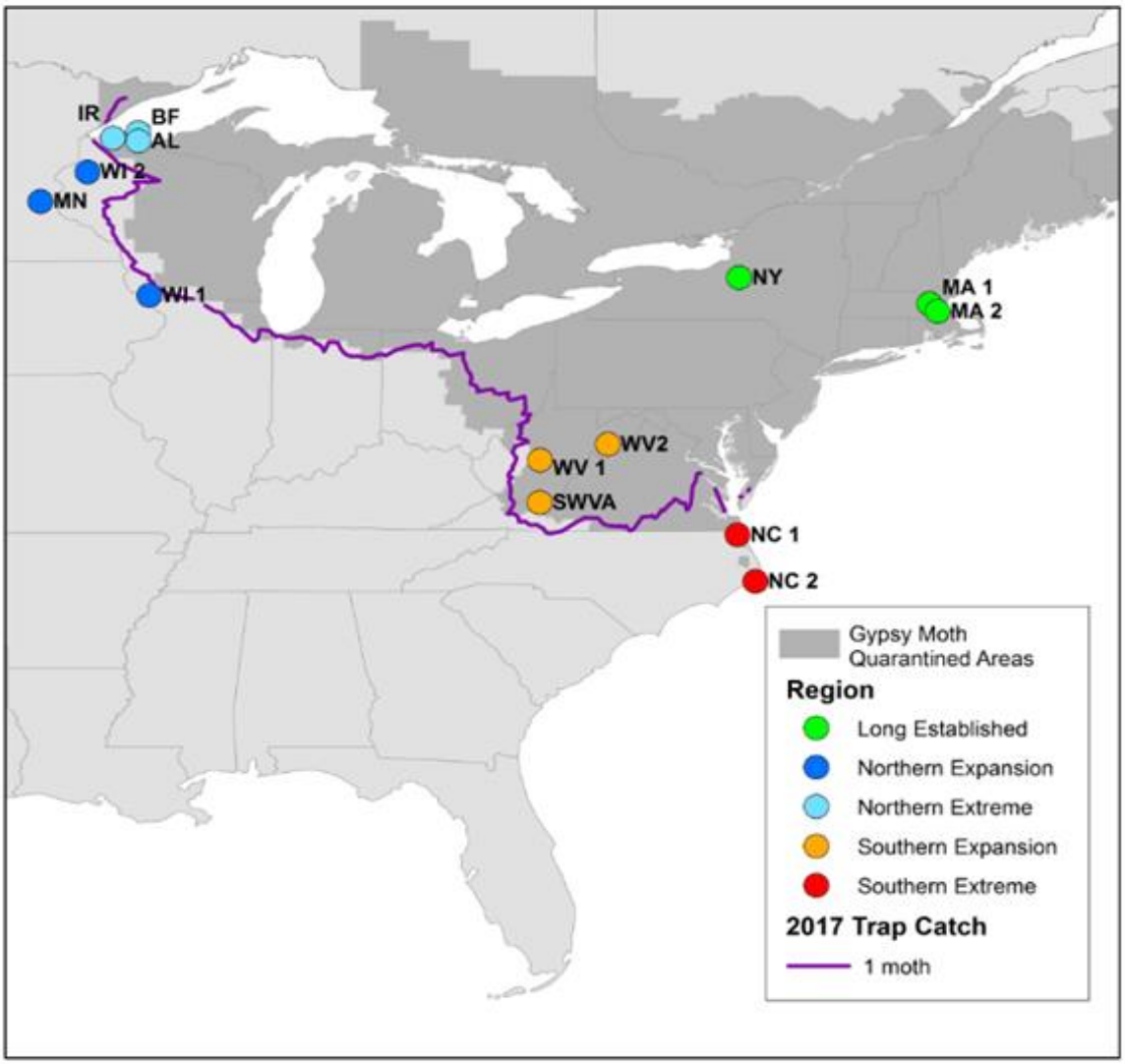

Figure 1) This shows the current range of L. dispar in North America. Each point represents a population being studied as part of the overall NSF project. Populations in my project include: AL, MA 1, NC 1, NC 2, NY, SWVA, WI 1, and WV 1.

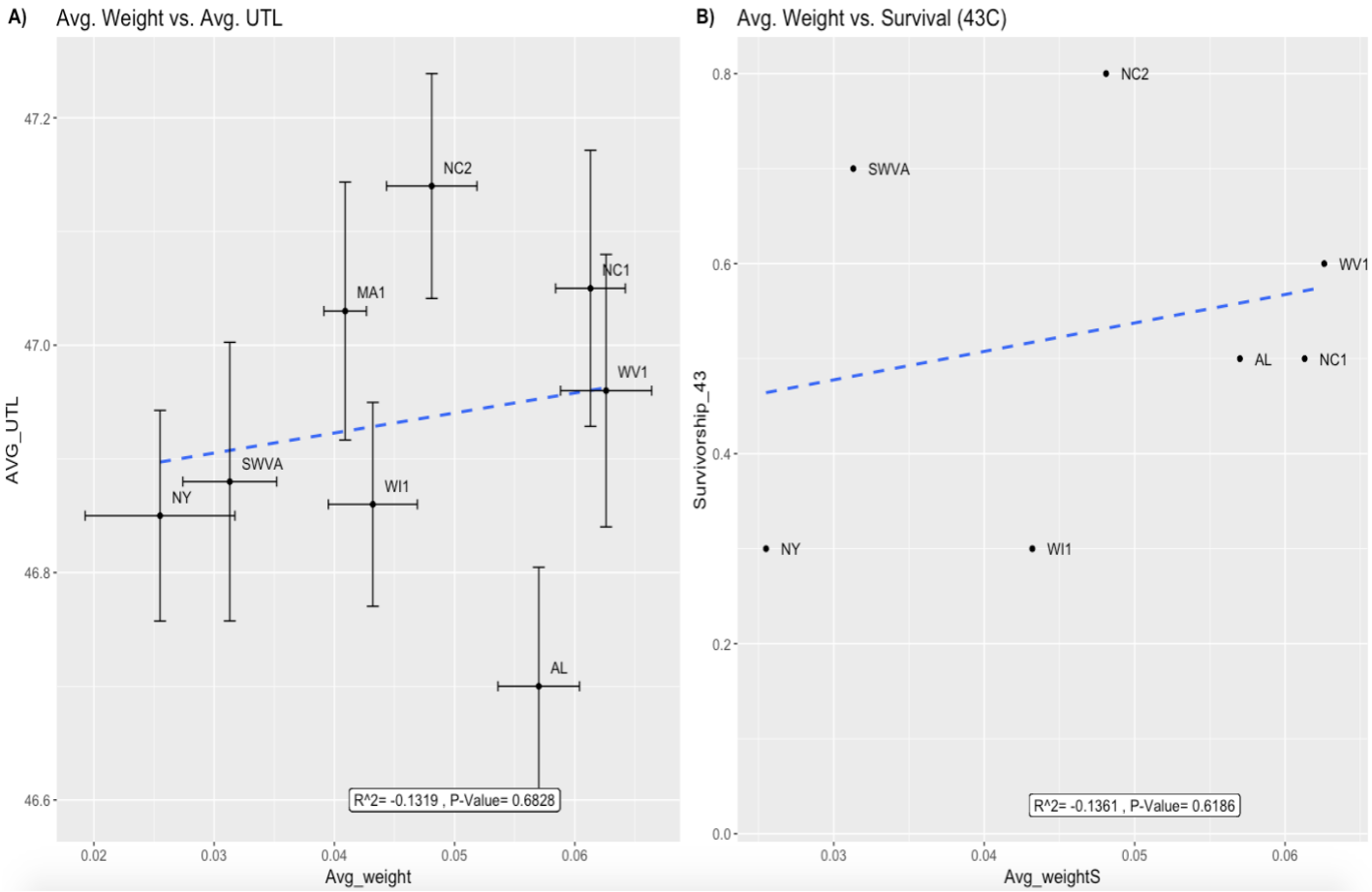

Figure 2) Graph A: relationship between weight and average population UTL. Graph B: relationship between weight and proportion of L. dispar larvae survival at 43C

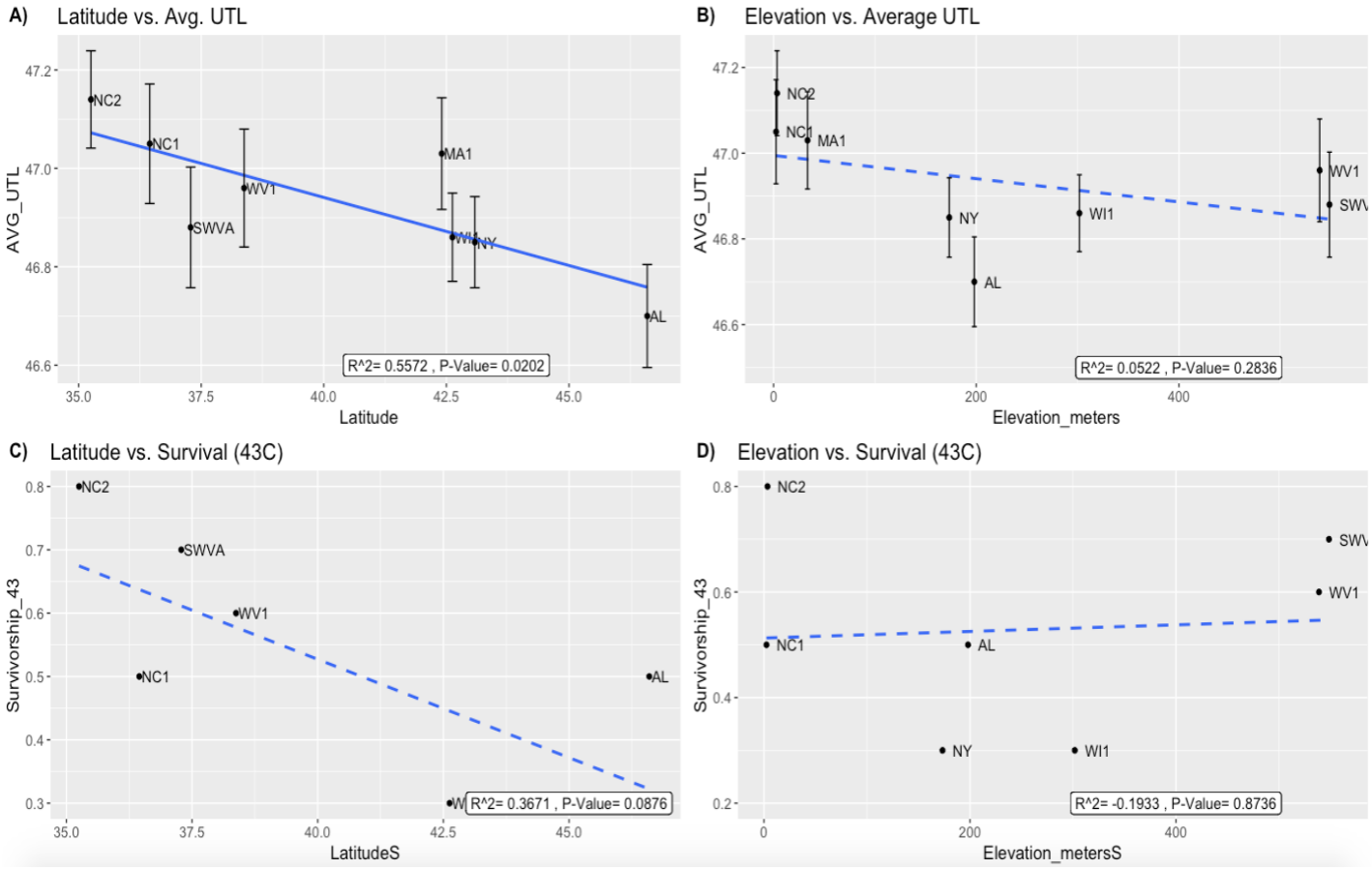

Figure 3) Graph A: relationship between latitude and average population upper thermal limits. Graph B: relationship between elevation and average population upper thermal limits. Graph C: relationship between latitude and average population survivorship at 43C. Graph D: relationship between elevation and average population survivorship at 43C. Dotted regression lines indicate relationships that were not statistically significant.

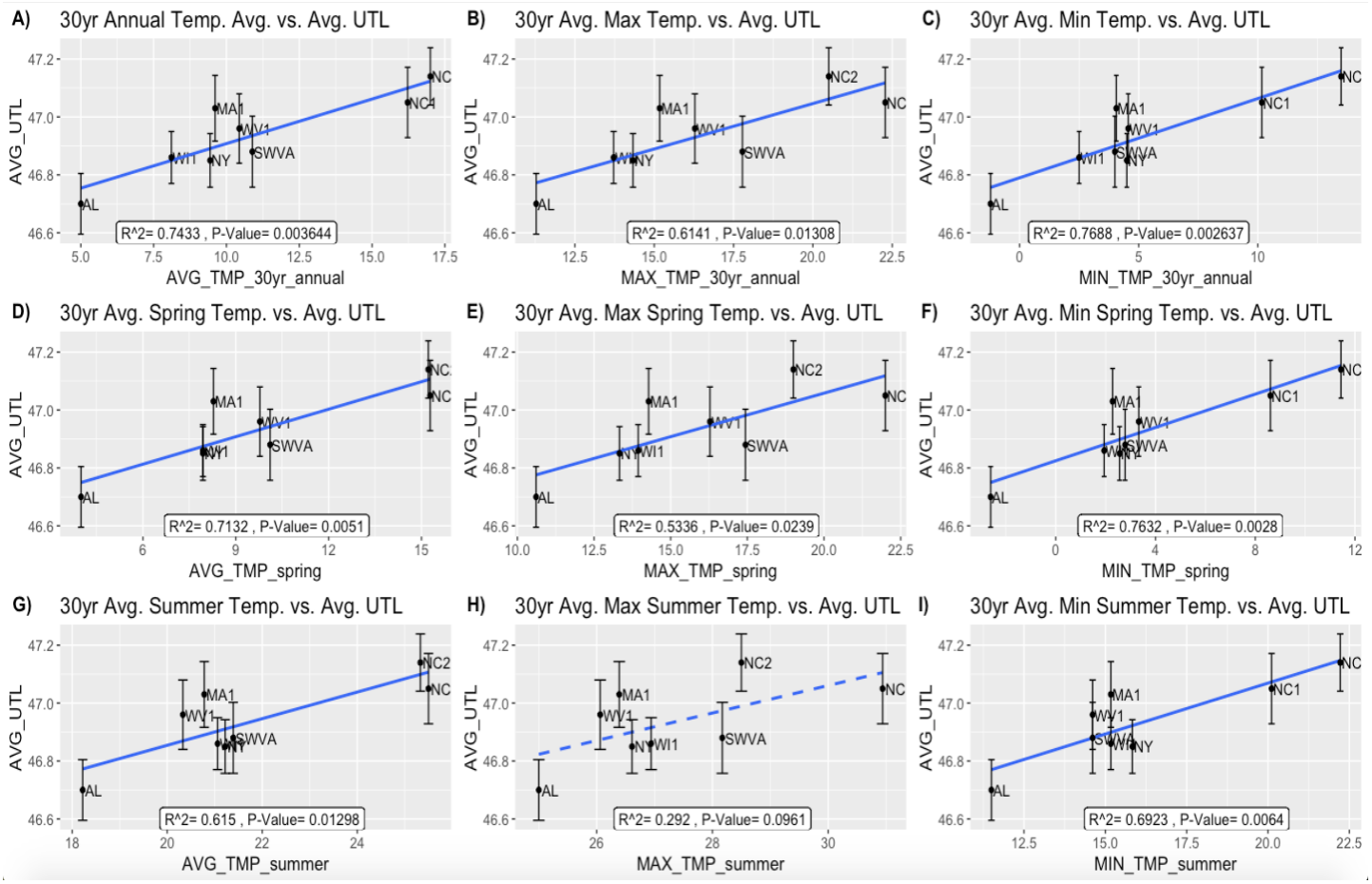

Figure 4) Results from linear regression analysis of Ramp treatment data. Graph A: relationship between 30 year annual average temperature and average population UTL. Graph B: relationship between 30 year average maximum temperature and average population UTL. Graph C: relationship between 30 year average minimum temperature and average population UTL. Graph D: relationship between 30 year average spring temperature and average population UTL. Graph E: relationship between 30 year average maximum spring temperature and average population UTL. Graph F: relationship between 30 year minimum spring temperature and average population UTL. Graph G: relationship between 30 year average summer temperature and average population UTL. Graph H: relationship between 30 year average maximum summer temperature and average population UTL. Graph I: relationship between 30 year average minimum summer temperature and average population UTL. Dotted regression lines indicate relationships that were not statistically significant.

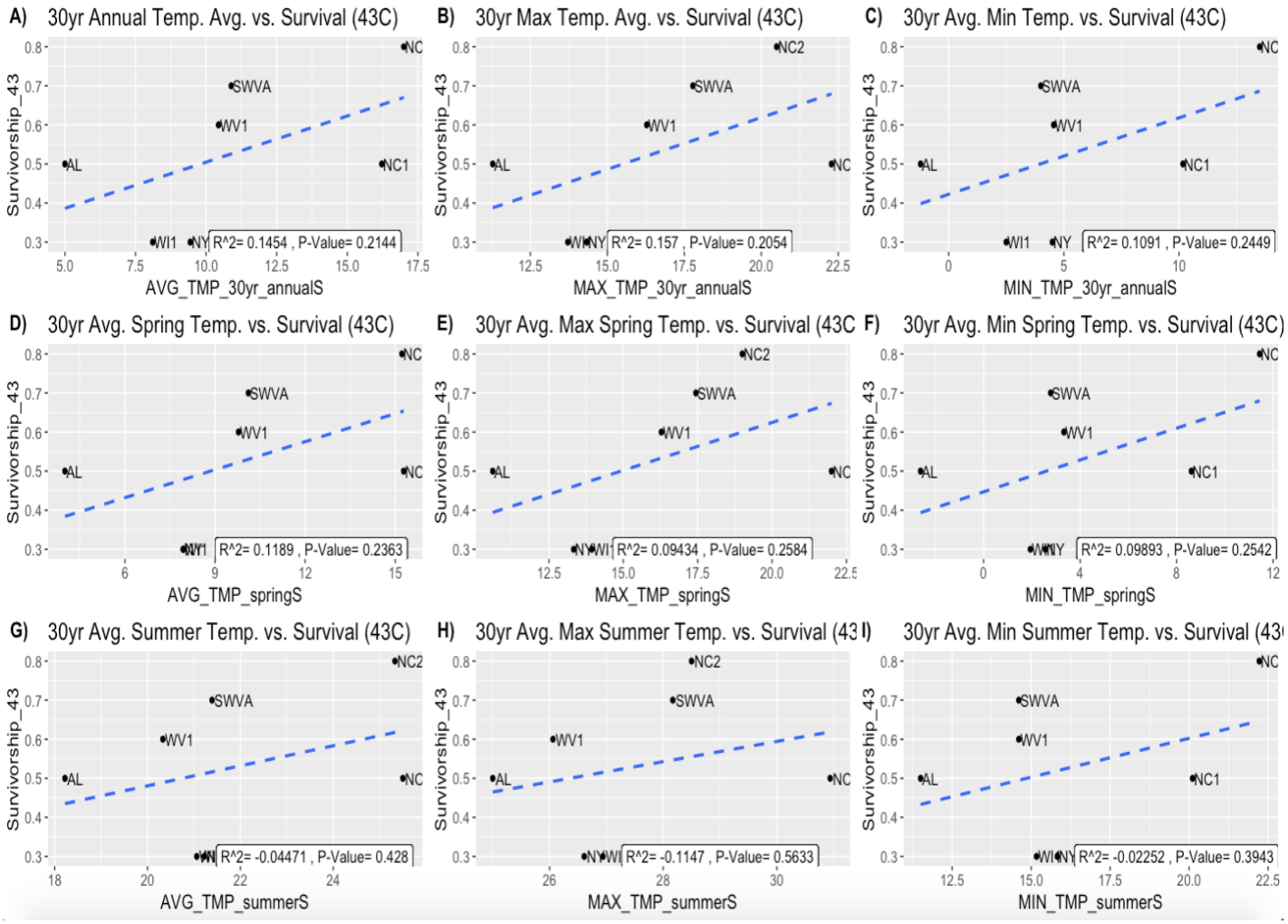

Figure 5) Results from linear regression analysis of soak trial data. Graph A: relationship between 30 year annual average temperature and Survivorship at 43C. Graph B: relationship between 30 year average maximum temperature and Survivorship at 43C. Graph C: relationship between 30 year average minimum temperature and Survivorship at 43C. Graph D: relationship between 30 year average spring temperature and Survivorship at 43C. Graph E: relationship between 30 year average maximum spring temperature and Survivorship at 43C. Graph F: relationship between 30 year minimum spring temperature and Survivorship at 43C. Graph G: relationship between 30 year average summer temperature and Survivorship at 43C. Graph H: relationship between 30 year average maximum summer temperature and Survivorship at 43C. Graph I: relationship between 30 year average minimum summer temperature and Survivorship at 43C. Dotted regression lines indicate relationships that were not statistically significant.